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Review

Structure, processing and properties of surfactant protein A

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Abstract

Surfactant protein A (SP-A) is a highly ordered, oligomeric glycoprotein that is secreted into the airspaces of the lung by the pulmonary epithelium. The in vitro activities of protein suggest diverse roles in pulmonary host defense and surfactant homeostasis, structure and surface activity. Functional mapping of SP-A using directed mutagenesis has identified domains which interact with surfactant phospholipids, alveolar type II cells and microbes. Recently developed genetically manipulated animal models are beginning to clarify the critical physiological roles for SP-A in the normal lung, and in the pathophysiology of pulmonary disease. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Surfactant protein A; Surfactant; Pulmonary host defense; Collectin; C-type lectin; Surfactant homeostasis

Contents

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1. Introduction

The alveolar surface of the lung is a vulnerable biological interface, subject to destabilizing surface forces that promote airspace collapse, and to continuous bombardment by inhaled particulates, pathogens and toxins. The survival of air breathing organisms is dependent on the presence of a multifunctional pulmonary epithelial lining substance called surfactant [1]. Surfactant is an oily mixture of proteins, phospholipids and neutral lipids that is secreted by alveolar type II cells and organized as a layer (or layers) at the air–liquid interface. The surfactant film both reduces surface pressures and actively participates in the clearance of foreign material. The most critical component of surfactant for airspace stability is dipalmitoylphosphatidylcholine (DPPC), a saturated phospholipid which accounts for virtually all of surfactant's surface tension lowering properties. Two hydrophobic surfactant proteins, surfactant protein B (SP-B) and surfactant protein C (SP-C), are critical for the adsorption and spreading of the surfactant film at the air–liquid interface (Chapter IX). Surfactant protein D, on the other hand, has in vitro activities that suggest a primary role in host defense (Chapter XV). The most abundant surfactant protein, surfactant protein A (SP-A) was first identified by King and Clements in 1972 [2]. Despite early discovery and intense investigation, the function of SP-A is perhaps the most controversial of the four surfactant proteins. Most studies have focused on the role of SP-A in surfactant structure and function, but there is increasing evidence that SP-A participates in pulmonary host defense. In this chapter, the structure and processing of SP-A will be reviewed and compared with other members of the collectin family of related proteins. The biochemical properties of SP-A will also be discussed, especially as they relate to alveolar type II cell and surfactant phospholipid interactions, with an emphasis on structure–function relationships. The host defense properties of SP-A will only be mentioned in the context of known structure–function relationships as they relate to binding, opsonization and clearance of microorganisms. For a more detailed discussion of the innate immune properties of SP-A, the reader is referred to Chapters XII and XIII.

2. Structure

2.1. Overview of SP-A structure and assembly

Surfactant protein A has been found in every vertebrate species that has been carefully examined, including in the lungs and swimbladders of some species of lungfish and goldfish [3,4]. Sequence comparison of the cDNAs for SP-A isolated from human [5], dog [6], rabbit [7], guinea pig [8], rat [9] and mouse [10] reveal extensive homology. The deduced primary structure of SP-A is characterized by four well defined structural domains (Fig. 1): a short N-terminal segment containing interchain disulfide

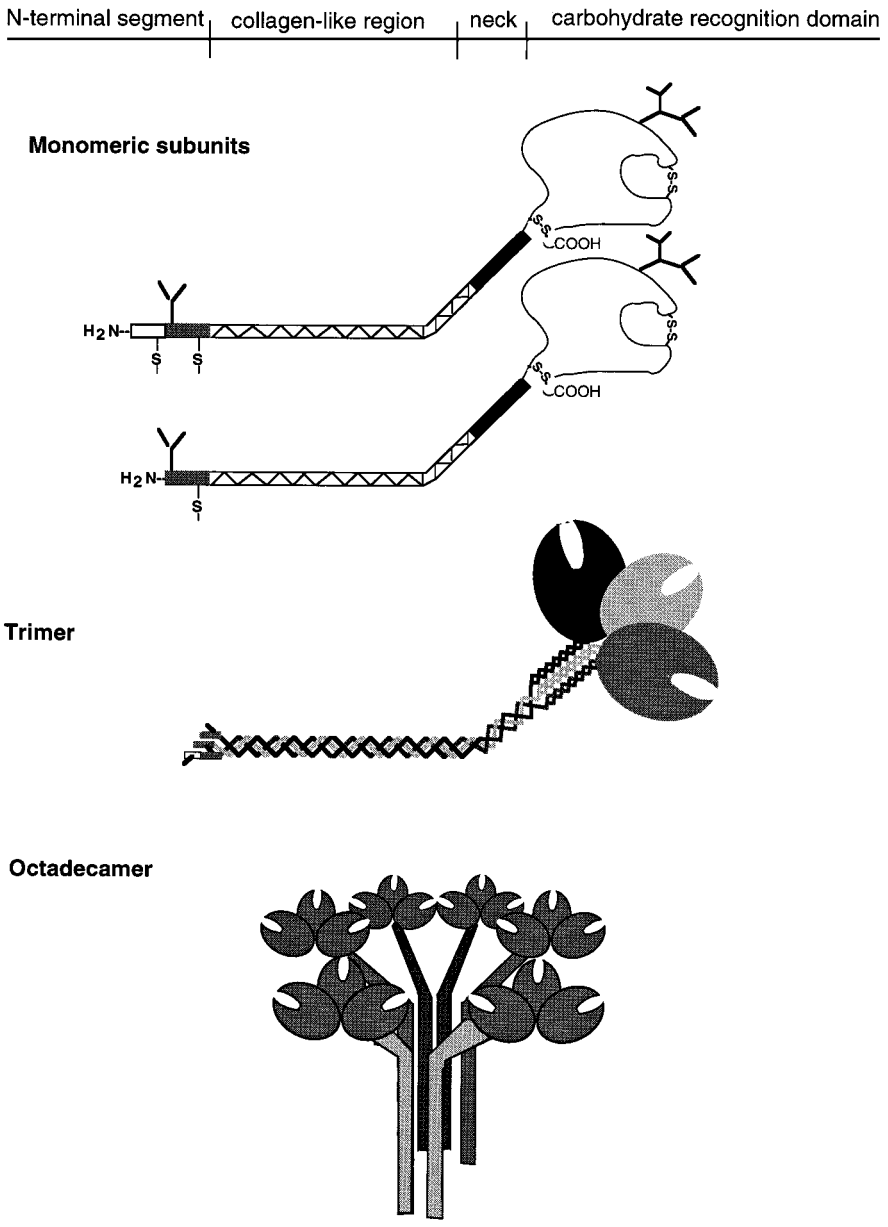

Fig. 1. Monomeric and oligomeric structure of SP-A. The deduced primary structure of SP-A predicts four major structural domains: a short N-terminal segment containing two intermolecular disulfide bonds, a collagen-like sequence of gly–*x*–*y* repeats, an acidic and hydrophobic neck domain and a C-terminal carbohydrate recognition domain. The neck domains of three SP-A monomers become associated as rigid α-helical coiled coils and the collagen-like regions fold into staggered collagen triple helices. Six trimeric subunits form the fully assembled, octadecameric molecule, which is stabilized by disulfide linkages in the N-terminal domains.

bonds, a proline-rich collagen-like region, a hydrophobic 'neck' domain and a carbohydrate recognition domain (CRD) with bears a high degree of homology to several members of the Ca^{2+}-dependent (C-type) mammalian lectins, especially mannose binding protein A (MBP-A) [11]. The combination of a collagen-like region and a C-type lectin domain are the defining structural features of the emerging collectin family of proteins, many of which have been shown to function as antibody independent opsonins [12]. In addition to SP-A, the other collectins include MBP-A [11], bovine conglutinin [13], CL-43 [14], and SP-D [15].

Surfactant protein A is recovered from the airspa-

ces of the lung by bronchoalveolar lavage. High-speed centrifugation of the lavage fluid sediments SP-A complexed with surfactant phospholipids. SP-A is most commonly purified from the pellet by butanol extraction [16] followed by adsorption to a monosaccharide-linked affinity matrix and size-fractionation by gel exclusion chromatography [41]. Purified SP-A migrates heterogeneously on SDS–PAGE under reducing conditions [17], due largely to variable glycosylation with sialic-acid containing complex carbohydrates at one [18] (human, dog, rabbit) or two (guinea pig, mouse and rat) [17,18] potential asparagine attachment sites. Under nonreducing conditions, the protein appears as a ladder of disulfide-linked multimers composed of up to six or more subunits [16,17].

Based on analogy with other collectins, the folding of SP-A in the endoplasmic reticulum likely proceeds from the C-terminal to N-terminal direction in a zipper-like fashion [19,20]. These studies predict that globular CRDs fold separately while the more N-terminal domains, the collagen-like region and the neck, become associated as trimeric helices [21]. The neck domain of SP-D forms a triple stranded α-helical nonstaggered bundle that functions as the nucleation site for the folding of the collagen-like domain into staggered triple helices [22]. The formation of collagen-like triple helices in SP-A is supported by circular dichroism studies [23–25], but the folding of the neck region has not been directly examined. The formation of N-terminal intermolecular disulfide bonds in SP-A probably occurs last, as it does in SP-D, after the folding of the collagen region is completed [20].

The apparent molecular mass of human SP-A in solution is reported to be 650 kDa by gel filtration analysis and sedimentation equilibrium studies [23], indicating the association of six trimers. Under the electron microscope, SP-A has a highly ordered structure that resembles a bouquet of tulips [25]. Taken together, the available data are consistent with an hexagonal, in-register array of trimeric units, which are laterally bound by strong hydrophobic (van Der Waals) forces and charged interactions through the first half of the collagen-like domain, and stabilized by inter- and intra-trimeric disulfide bonds at the N-terminus (Fig. 1) [25]. This structure is very similar to that of C1q (which is not a collectin) [26] and MBP-A. The collectins with more elongated collagen-like regions either form cruciform structures (SP-D and conglutinin) or simple trimers (CL-43 and MBP-C).

2.2. Comparative structure of individual domains of SP-A

2.2.1. The N-terminal segment

The N-terminal segment of SP-A is 8–11 amino acids in length, depending on the species and variation in N-terminal processing (see below). There are two Cys residues present in this region in SP-A from all species examined except dog, which contains only one. Both Cys have been shown to participate in interchain disulfide bond formation in rat SP-A, and to be required for multimer formation [27]. The pattern of disulfide linkage is most likely a symmetrical 1–1, 2–2 pattern, based on the elution pattern during N-terminal protein sequence analyses. There are also two Cys present in the N-terminal segment of all other collectins [12] but in the case of MBP-C disulfide bridges appear to be asymmetrical and heterogeneous (1–1, 2–2, 1–2 or 1–2, 1–2, 1–2) [28]. There are several highly conserved charged and hydrophobic amino acids flanking the second Cys in the SP-As from different species, but the N-terminal segment does not bear any significant homology to the other collectins or to other proteins in GenBank. The interchain disulfide bond formed by the Cys adjacent to the collagen-like region of rat SP-A (Cys^6) has been shown to be required for interactions with surfactant lipids and alveolar type II cells [29] (see below), but preliminary data indicate that the more N-terminal Cys^{-1} is not critical for these functions [30].

2.2.2. The collagen-like region

The collagen-like region of SP-A contains a series of 23 (human,baboon) or 24 (rat, rabbit, dog, guinea pig) gly–*x*–*y* repeats. The deduced primary structures of human and rat cDNAs predict Pro in the *x* position of 4 or 5 of the repeats, clustered near the midpoint and the C-terminal end of the collagen domain. Prolines are also predicted in the *y* position of 15 or 16 of the repeats, most or all of which are hydroxylated based on partial N-terminal protein sequencing analysis [9]. The presence of hydroxyproline in colla-

gens greatly enhances the stability of the collagen helix [31]. The first half of the collagen-like domain of SP-A, which is the most highly conserved region in the molecule, contains a series of alternately positively and negatively charged amino acids (+/−, +/−). This charge pattern is conserved in the other collectins as well, except that Gln occupies the second 'positive' position in CL-43. There are also alternating charged amino acids in the second half of the collagen domain of SP-A, but the order is reversed (−/+, −/+). These charged segments may contribute to the stability of the triple helix, to binding to a common collectin receptor [32], and/or to association of SP-A with soluble ligands [33–35]. The two charged motifs are separated by an interruption in the reiterative tripeptide sequence after the 12th repeat. This 'hinge region' introduces a visible 60° bend into the collagen domain which tilts the globular heads away from the core in the fully assembled molecule [25]. An interruption in the collagen region of the structurally related molecule C1q also introduces a bend into the domain [36], but a similar break in the collagen sequence in MBP-A not produce any obvious structural change [37]. The other known collectins contain continuous collagen-like regions. The collagen-like domain is important for binding to at least one known SP-A receptor [38] and for specific inhibition of surfactant secretion from isolated type II cells [29], but it is not yet clear if the collagen region participates in direct protein–protein interactions or whether it is primarily a scaffolding that amplifies the ligand binding activities of other domains of the molecule (e.g., the CRD).

2.2.3. The neck region

Constructs composed of the neck+CRD of SP-A are trimeric in solution, based on crosslinking studies (F. McCormack, unpublished observations) and gel exclusion chromatography [24]. Like all collectins, the stretch of 38–40 amino acids between the collagen-like region and the lectin domain of SP-A contains hydrophobic amino acids that can be roughly organized into heptad repeats. It is very likely that the polypeptide strands in the neck region of SP-A fold separately into rigid α-helical coils that are noncovalently associated by strong hydrophobic (van Der Waals) forces, as does the corresponding region of SP-D [22], although this has not been directly examined. This model predicts two hydrophobic faces of each SP-A polypeptide chain that are potential association domains for adjacent chains, and four faces containing charged and polar residues that are directed toward the solvent. There are two highly conserved positively charged residues at the midpoint of this otherwise very acidic region (e.g., His^{95}, Lys^{96} in rat SP-A). In addition to its critical role in protein folding, the neck domain may orient the CRDs of SP-A trimers for selective ligand binding. The solution of the structure of the MBP-A trimer revealed that each CRD is spaced approximately 50 Å from the other two [21]. Hydrophobic amino acids at the C-terminal end of the neck domain of MBP-A form the interface with the CRD, and sequence variations in this region of the collectins may play a role in determining the alignment of the CRDs [21]. There is some evidence that cooperative interactions between the neck and CRD contribute to SP-A interactions with type II cells [39], but it is not clear if the neck domain of SP-A has any direct ligand binding roles.

2.2.4. The carbohydrate recognition domain

The CRDs from animal lectins can be broadly classified into those that preferentially bind galactose or *N*-acetylgalactosamine and those that bind mannose or *N*-acetylglucosamine [40]. The CRD of SP-A, a member of the mannose subgroup, has been shown to mediate a variety of interactions including modulation of alveolar type II cell functions, binding and aggregation of phospholipids, and non-immune recognition of bacterial, viral and fungal organisms. This region contains approximately 115 amino acids including four conserved Cys which form two intramolecular disulfide loops, as well as 10 additional invariant and 18 highly conserved amino acid residues common to the C-type lectins [41]. There are at least two Ca^{2+} binding sites in the CRD of SP-A [42,43], and all reported CRD functions are Ca^{2+}-dependent. There are only two tryptophans in the CRDs of all species which have been characterized to date [8], and both are located near the Ca^{2+} binding sites predicted by comparison with the crystal structure of MBP-A (see below). In this position the tryptophans can be used as reporters to detect conformational changes in the region. The binding of Ca^{2+} to the CRD of SP-A results in an increase in

intrinsic fluorescence and a shift in the emission maxima toward shorter wavelengths, indicating translocation of tryptophan to a more hydrophobic environment [42,44]. This conformational shift enhances resistance to protease digestion, probably by burying potential protease cleavage sites in the core of the protein [42]. Mutagenesis studies suggest that the carbohydrate binding site of SP-A colocalizes with a major Ca^{2+} binding site [39,45,46], and that amino acid substitutions that eliminate conformational shifts also interfere with the karacteristic ligand binding functions of the CRD [46,47].

X-Ray crystallographic studies of the CRDs of natural and mutant recombinant C-type lectins have elucidated the intermolecular forces that determine carbohydrate binding in this protein family [48–51]. The first C-type lectin and the only collectin structure that has been solved is MBP-A complexed with an asparaginyl oligosaccharide (Fig. 2) [52]. Analysis of the acceptor and ligand together clarified the relationship of atomic components of the penultimate mannose residue of the oligosaccharide to the amino acids and Ca^{2+} ions in the carbohydrate binding site of the protein [49]. The carbohydrate binding site is located in a large loop that arises from a tight core domain composed of two helices, two beta strands and two beta sheets. The loop contains three Ca^{2+} binding sites, one located in the sugar binding site (denoted #2 Ca^{2+}), and two located closer to the origin of the loop (#1 and #3 Ca^{2+}). The #3 Ca^{2+} binding site was thought to be artifactual. The diffraction analysis revealed that the carbohydrate is bound by network of Ca^{2+} coordination bonds and hydrogen bonds [40]. The #2 Ca^{2+} ion was complexed with oxygen atoms from the side chains of Glu^{185}, Asn^{187}, Glu^{193}, Asn^{205}, and Asp^{206} (see Fig. 2). The 3′ and 4′ equatorial hydroxyl groups of mannose also formed Ca^{2+} coordination bonds, as well as hydrogen bonds with amine and carbonyl side chains of Glu^{185}, Asn^{187}, Glu^{193} and Asn^{205}. Subsequent site-directed mutagenesis studies confirmed crystallographic evidence that van der Waals interactions between sugar and the imidazole ring of His^{189} and carbon atoms of Ile^{207} also contributed to binding [53]. Thus, the binding of mannose to individual CRDs of MBP-A is due to a collection of relatively weak interactions. The affinity of MBP-A for ligands is greatly increased by the simultaneous binding of multiple CRDs of each oligomer to the sugar, lipid or protein target [54].

The solution of the structure of C-type lectin–oligosaccharide complexes has also demonstrated that carbohydrate binding specificity is determined by structural characteristics of both the carbohydrate and the protein [40,52]. Sugars exhibiting the preferred axial and equatorial arrangement of key hydroxyl groups 'fit' into carbohydrate binding sites in which carbonyl and nitrogen containing amino acid side chains are presented in the correct context for hydrogen bond formation. The C-type lectins which bind to mannose prefer sugars with equatorial hydroxyl groups at the 3- and 4-positions of the hexose ring, while lectins which bind galactose recognize sugars with an axial 4-hydroxyl. Drickamer provides elegant confirmation of the structural elements of MBP-A that confer selectivity [55]. He noted that C-type lectins which bind to mannose contain a conserved Glu–Pro–Asn (EPN) sequence in the carbohydrate binding site of MBP-A, while related protein family members that exhibit selective binding to galactose contained a Gln–Pro–Asp (QPD) motif in the corresponding region of the CRD. The construction of a mutant recombinant MBP-A containing a QPD substitution at the EPN locus converted the carbohydrate binding preference from mannose to galactose, as was predicted. Other structural features have also been shown to play a role in the discrimination between favored and unfavored ligands, such as steric exclusion. Finally, the spatial arrangement of multiple CRDs on the rod like scaffolding of the collectins enable them to bind specifically to microorganisms. Solution of the trimeric structure of MBP-A revealed that the spacing of the CRDs in MBP-A precludes multivalent binding to a typical mammalian high mannose oligosaccharides, but it is optimal for avid binding to the repetitive sugar arrays on the surfaces of pathogenic organisms [21].

3. Processing

3.1. Tissue distribution

There are two transcribed genes for human SP-A on the long arm of chromosome 10 [56,57], which encode isoforms containing minor amino acid differ-

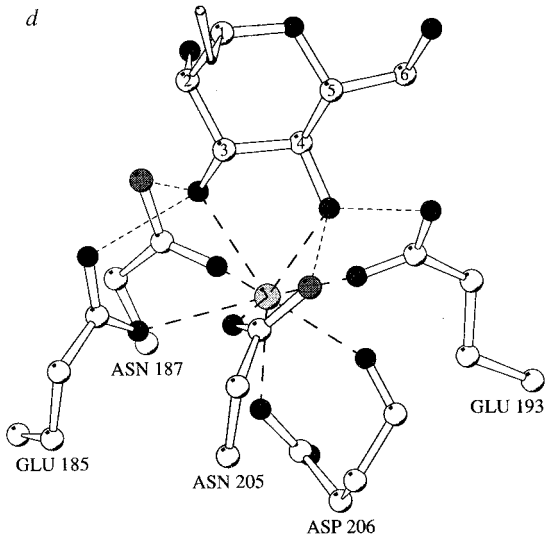

Fig. 2. The Ca^{2+} and carbohydrate binding site of MBP. X-Ray diffraction analysis of an MBP-A–asparaginyl oligosaccharide complex revealed the relative positions of Glu 185, Asn 187, Glu 193, Asn 205 and Asp 206 of the carbohydrate binding site of the CRD of MBP-A, a Ca^{2+} ion and the terminal mannose residue of the ligand. The Ca^{2+} ion is anchored by coordination bonds with oxygen atoms from all five amino acids. The 3- and 4-hydroxyl groups of the mannose residue are bound by Ca^{2+} coordination bonds and hydrogen bonds with amine and carbonyl side chains of Glu 185, Asn 187, Glu 193 and Asn 205. Not shown are hydrophobic interaction between the sugar and His 189 and Ile 207. The Ca^{2+} and carbohydrate binding site of SP-A is modeled based on the MBP structure. (Drawing from Ref. [49].)

ences in the protein. The baboon also has two genes [58], but only one gene has been found in all lower species that have been examined. Surfactant protein A mRNA and protein are expressed in epithelial cells of the distal airspaces, including alveolar type II cells and the nonciliated bronchiolar cells (Clara cells) of the terminal bronchioles and conducting airways (especially in rodents) [59,60]. SP-A mRNA is also found in the serous glands of the proximal human trachea [59]. Immunohistochemical staining detects SP-A in the endocytic compartment of macrophages but de novo synthesis does not occur within the phagocyte. Western-blot analyses have suggested the presence of SP-A in rat intestinal epithelia [61], human and rat mesentery [62] and human inner ear [63], although the precise identity of the immunoreactive proteins has not been determined.

3.2. Signal peptidase cleavage and N-terminal processing

SP-A is synthesized as a preprotein with a leader sequence of 17–28 amino acids, which is cleaved from the nascent peptide as it is translocated into the endoplasmic reticulum. Elhalwagi et al. recently reported two alternative N-termini in rat SP-A, suggesting heterogeneity in N-terminal processing [27]. They found that rat SP-A is composed of a short variant that has the predicted N-terminus (Asn^1–Val–Thr–Val^4), and a less abundant (approx. 20%) variant that contains three additional amino acids at the N-terminus, including an additional Cys (Ile^{-3}–$Lys–Cys^{-1}$–Asn^1–Val–Thr–Val^4). Expression of a full-length cDNA for rat SP-A in eucaryotic cells resulted in the production of recombinant proteins representing both variants, in the same 80:20 (short:long) ratio that was observed for the natural SP-A. This result indicated that a tissue-specific protease was not required for the alternative cleavage. Deletion mutagenesis that varied the amino acid sequence downstream of the cleavage region altered the abundance of the short and long variants, consistent with the known sensitivity of signal peptidase to the context of the amino acid sequence near the cleavage site. Collectively, the data suggests that N-terminal microheterogeneity arises by differential use of alternative signal peptidase cleavage sites.

Human SP-A also has variable N-termini [27], including two cysteinyl variants, but SP-A from other species has not been examined. The identification of a second Cys at the N-terminus of SP-A is consistent with the primary structure of the other collectins that have been described to date, which all have two N-terminal Cys [12]. The structural features of the leader sequence and N-terminal sequence that lead to alternative cleavage are not known, but possibilities include cotranslational disulfide bond formation at Cys −1 of the nascent peptide or the use of alternative translation initiation sites (Met-20 or Met^{-}-29 in rat SP-A). Another protein which has been shown to exhibit microheterogeneity due to alternative signal peptidase cleavage at the N-terminus is interferon ω1 [64], a recently discovered protein with an unknown physiological function that is structurally related to interferon α and β. Interestingly, like SP-A, this molecule also contains Cys residue near the primary

cleavage site and a second, in-frame methionine in the leader peptide which might potentially function as a second translation initiation site. Differential use of alternative translation initiation sites as a mechanism to regulate expression of protein isoforms has been described [65].

3.3. Post-translational modification, sorting and trafficking

After synthesis, SP-A is extensively modified in the rough endoplasmic reticulum (ER) and Golgi, in a sequence that appears to parallel that of the fibrillar collagens [31]. Mammalian collagens are hydroxylated [23,67] in the endoplasmic reticulum by prolyl hydroxylase [68], a heterotetrameric enzyme composed of α and β subunits ($\alpha_2\beta_2$) that catalyzes the formation of 4-hydroxyproline. This enzyme also catalyzes strand alignment and the assembly of thiol-dependent multimers through the activity of its β subunit, which has been found to be identical to the enzyme protein disulfide isomerase [69]. Most of the folding and assembly of SP-A occurs before the protein exits the endoplasmic reticulum (ER). SP-A is also cotranslationally glycosylated with mannose-rich, endoglycosidase H (endo H) sensitive carbohydrates in the ER. The acquisition of endo H resistance is used as a marker of entry of SP-A into the Golgi apparatus [18]. Late post-translational carbohydrate modifications in this organelle include sialylation [16] and sulfation [66], which contribute to charge heterogeneity of the molecule on electrophoretic analysis. The complex folding, assembly and processing of SP-A likely contributes to the relatively slow rate at which SP-A traverses the secretory pathway [70].

The structural requirements for proper trafficking have been explored using metabolic inhibitors and heterologous expression of recombinant proteins in eucaryotic cells. *N*-Linked carbohydrate is not required for intracellular trafficking, since elimination of *N*-linked glycosylation sites [71] or incubation with specific inhibitors of glycosylation [18,72] do not block secretion of the protein. Secretion from eucaryotic cells is preserved despite genetic disruption of either N-terminal interchain disulfide bond of rat SP-A [29,30], but blocking disulfide bridge formation at both locations results in intracellular retention (F. McCormack, unpublished observation). Inhibition of hydroxylation or incorporation of cis hydroxyproline results in accumulation of SP-A in the endoplasmic reticulum, presumably due to disrupted assembly of the SP-A multimer [70,72]. However, underhydroxylation of the collagen region that occurs when recombinant SP-A produced in invertebrate cells does not block secretion [71]. Similarly, partial or complete deletion of the collagen-like region does not prevent secretion from insect cells [29] or mammalian (CHO) cells (F. McCormack, unpublished observation). Interestingly, the removal of the collagen-like region appears to relax the secretory requirement for interchain linkage, since a construct containing only the neck and CRD of SP-A (SP-$A^{\Delta N1-P80}$) is secreted from insect cells despite the absence of intermolecular disulfide bonds [27]. Mutations of the neck region of SP-A are generally poorly tolerated. Point substitutions, partial deletions and chimeric substitutions of the neck region with corresponding portions of other collectins typically results in rapid intracellular degradation and/or retention (F. McCormack, unpublished observation), probably reflecting the importance of this domain in the orchestration of protein folding. In contrast, more than 15 CRD mutant SP-As, including those containing point mutations in Ca^{2+} and carbohydrate coordinating residues [46,73] and large substitutions spanning the putative lipid and carbohydrate ligand binding domains [74,75], traverse the secretory pathway and can be recovered from the extracellular compartment. There are some exceptions; for example, deletion of the Cys^{204}–Cys^{218} region of rat SP-A caused the protein to be retained [39]. These data provide evidence that N-terminal interchain linkage and a properly folded collagen-like domain are required for proper intracellular routing, but N-linked oligosaccharides and the integrity of the CRD may be less critical for trafficking of SP-A through the secretory pathway.

Surfactant phospholipids and the hydrophobic surfactant proteins exit the Golgi and are routed through the multivesicular body to the lamellar body [76]. In response to the appropriate stimulus, they are secreted together in a regulated fashion. Although small amounts of SP-A are found in the periphery of lamellar bodies by immunoelectronmicroscopy [77], it is not clear that SP-A reaches the

lamellar body by the biosynthetic route as do the other surfactant components. In fact, the lopsided distribution of SP-A between the extracellular and intracellular surfactant compartments suggests that SP-A is secreted through a pathway that bypasses the lamellar body. SP-A makes up approx. 50% of total protein in isolated surfactant but only about 1% of total protein in lamellar bodies [78], which correlates with the abundance of SP-A in tubular myelin and paucity of SP-A in lamellar bodies detected by immunoelectron microscopy [79]. Metabolic labeling studies also support the existence of a second pathway. In preterm ventilated lambs pulsed with ^{35}S, SP-A appears in lavage fluid but not in LB in the first 2 h, and then gradually appears in the lamellar body [80]. It is possible that SP-A is secreted into the alveolar space by at two or more routes; via a regulated pathway that includes the lamellar body and a constitutive pathway that bypasses that compartment [80,81]. Alternatively, newly synthesized SP-A may be directly secreted and all SP-A within the lamellar body may reach the organelle by endocytosis of surfactant components from the extracellular space.

Table 1
Putative biological functions of SP-A

Surfactant homeostasis
Receptor-mediated inhibition of surfactant secretion from type II cells [118–120]
Receptor-mediated enhanced uptake of surfactant phospholipids by type II cells [152,153]
Surfactant biophysical activity
Enhanced phospholipid adsorption to the monolayer [109]
Prevention of protein inhibition by proteinaceous pulmonary edema [84,107,108]
Maintenance of surfactant aggregate structure, especially tubular myelin [83,99]
Maintenance of pool of surface active, large (dense) surfactant aggregates [84,100,103]
Phospholipase A_2 inhibition [148,149]
Scavenger functions in alveolar hypophase
Myosin clearance [151]
Lipopolysaccharide clearance and/or regulation of activity [33,34]
Host defense functions
Microbial binding and aggregation [47,87,154,155]
Macrophage activation and chemotaxis [156–160]
Enhanced microbial phagocytosis and killing [82,87,122,158,161–163]
Antiproliferative and antiinflammatory effects on lymphocytes [35,164,165]

Table 2
Biological ligands for SP-A

Surfactant phospholipids [111,166,167]
Dipalmitoylphosphatidylcholine
Phosphatidylcholine
Sphingomyelin
Pulmonary glycolipids [98,168]
Lactosylceramide
Galactosylceramide
Glucosylceramide
Asialo-G_{M2}
Proteins
Phospholipase A_2 [147–149]
Major surface glycoprotein of *P. carinii* [47,88]
Outer membrane protein of *H. influenza* [87]
Myosin [151]
Annexin IV [150]
SP-A receptors:
210 kDa SP-R (Chroneos) [38]
32 kDa SPAR (Strayer) [124,126,169]
126 kDa C1qRp (Nepomuceno) [130]
53 kDa C1qR (Malhotra) [131]
55 kDa BP55 (Wissel, Stevens) [127,128]

4. Properties

4.1. Background

Formulation of hypotheses about the functions of SP-A (Table 1) have been largely based on: (1) homologies with other proteins such as MBP-A, (2) ligand binding properties (Table 2) and measurable in vitro 'biological' activities (Table 3), (3) analyses of SP-A in pathological states in humans and experimental animals, and most recently (4) phenotypic characterization of genetically engineered animal models. There have been no reports of naturally occurring mutations in SP-A, such as those that have been useful in characterizing the function of other lung proteins (e.g., α1-antitrypsin, CFTR). The first decade of SP-A research was focused primarily on the role of the protein in surfactant homeostasis

Table 3
Reported in vitro activities of SP-A

Ca^{2+}-dependent self-aggregation [170]
Carbohydrate binding to simple sugars and polysaccharides [85,86]
Lipid binding
Ca^{2+}-dependent lipid aggregation [167,171]
pH-dependent lipid aggregation [46,172]
Enhanced surface activity of surfactant films [109]
Reduced inhibition of surfactant activity by foreign proteins [107,108]
Reconstitution of tubular myelin from surfactant components [99]
Inhibition of conversion of large to small surfactant aggregates [100,103]
PLA_2 inhibition [147–149]
Type II cell receptor binding [173–175]
Self internalization into type II cells [176,177]
Effects on cellular signalling pathways [125,178]
Effects on elaboration of cytokines and growth factors from type II cells [179]
Inhibition of surfactant secretion from isolated type II cells [118–120]
Enhancement of surfactant uptake into isolated type II cells [152]
Alveolar macrophage receptor binding [38,130–132,140,158, 180–184]
Enhanced alveolar macrophage chemotaxis [160]
Alveolar macrophage activation, elaboration of toxic oxygen species, NO etc. [156–159,185]
Stimulation of release of cytokines, growth factors, proteases etc. from alveolar macrophages [141,186,187]
Microbial binding, osponization, phagocytosis [122,129,154,158,161,163,188–191]
Lipopolysaccharide binding/regulatory molecule [33,34]
Lymphocyte receptor binding [35]
Reduced lymphocyte proliferation and cytokine expression [165]

and function, with a lesser emphasis on the putative host defense properties of the protein. For many laboratories, the prioritization of these two lines of lines of investigation has become inverted in the past several years, in part due to evidence of an immune defect in the SP-A knockout mouse ([82,83]; see Chapter XVI). Recent evidence of subtle surfactant dysfunction in this model suggests that SP-A will ultimately prove to have both surfactant and antimicrobial functions [84], the relative importance of which will become increasingly apparent as these and other genetically engineered animal models are more completely characterized. Other chapters in this series will address the host defense roles of SP-A (Chapter XIII) and the interactions of SP-A with phagocytes and epithelial cells (Chapter XII). The discussion below will therefore focus primarily on the relationships between the structure and function of SP-A, especially in the context of interactions with surfactant lipids and alveolar type II cells.

4.2. Binding of SP-A to monosaccharides and sugars associated with glycoproteins and glycolipids

The carbohydrate recognition domain of SP-A mediates the selective recognition of pathogenic organisms and the binding to several soluble and membrane-associated ligands. The lectin activity of SP-As was first suggested by Drickamer et al. [11], based on that observation that SP-A C-terminal sequences were 30% identical with rat MBP-A [5]. Haagsman et al. subsequently confirmed that SP-A binds to a variety of monosaccharide ligands, including mannose, glucose, galactose and L-fucose, via the C-terminal domains of the protein [85]. Better assessment of the selectivity of SP-A for sugars was obtained using a solid phase enzyme-linked lectin binding assay [86]. The ability of various monosaccharides to compete for binding of SP-A to mannan, a yeast-derived polymer of mannose, was found to follow the rank order: *N*-acetylmannosamine > L-fucose, maltose > glucose > mannose. Galactose, D-fucose, glucosamine, mannosamine, galactosamine, *N*-acetylglucosamine and *N*-acetylgalactosamine did not inhibit mannan binding. This pattern of ligand preferences was very similar to MBP-A, except that binding of MBP-A to mannan was strongly inhibited by *N*-acetylglucosamine.

Because there is no three-dimensional structural data available for SP-A, MBP-A is used as a model for SP-A–carbohydrate interactions. The amino acids Glu^{195}, Arg^{197}, Glu^{202}, Asn^{214} and Asp^{215} of rat SP-A correspond to the MBP-A coordination set for Ca^{2+} and carbohydrate binding. Alanine substitutions for each of these residues reduced the binding to both

carbohydrate-linked beads and Ca^{2+}, except substitution at Arg^{197} which had no effect [46]. Mutagenesis experiments were performed in which the Glu^{195}–Pro^{196}–Arg^{197} (EPR) sequence of rat SP-A was substituted with Gln^{195}–Pro^{196}–Asp^{197} (QPD) [45]. As was predicted based on the results of similar experiments performed with MBP-A [55], these mutations increased the binding of SP-A to galactose. These experiments confirmed that the carbohydrate binding site of SP-A maps to the region predicted by homology with MBP-A and confirmed a role for the EPR sequence in the determination of sugar binding specificity of SP-A. The alanine mutant SP-As and the QPD protein have proven to be useful tools to dissect the role of the CRD in several of the reported functions of the protein (see below).

SP-A binds specifically to high mannose carbohydrates that are commonly present on the surface of microbes but which are not usually found on mammalian cells. In bacterial organisms, these sugars are most likely components of lipooligosaccharides or capsular oligosaccharides [87]. The only reported microbial glycoprotein ligand for SP-A that is known to bind via a lectin–carbohydrate interaction is the major surface glycoprotein (MSG) of *Pneumocystis carinii* (Pc) [88]. Pc causes a severe pneumonia in immunosuppressed humans and laboratory animals that is consistently associated with an elevation in the alveolar level of SP-A [89]. The MSGs are a closely related family of abundant cell surface glycoproteins that are thought to be major targets for the cellular and humoral immune defenses of the host [90–92]. There are up to 100 similar but distinct copies of MSG in the Pc genome [93], that are selectively expressed by translocation to a telomeric expression site called the upstream conserved sequence (UCS) [94]. The shuttling of different genes in and out of the UCS expression site is thought to result in antigenic variation of MSGs that are presented on the surface of the organism [95,96]. SP-A may function as a broad spectrum antibody independent opsonin that assists in the clearance of Pc by lectin mechanism that is not dependent on the recognition of changing peptide epitopes. In 1992, Zimmerman et al. reported that MSG was a ligand for SP-A [88]. This conclusion was based on the finding that SP-A bound to whole Pc in a Ca^{2+}- and carbohydrate-dependent manner, and to a protein species in membrane preparations of Pc that had the mobility of MSG on two-dimensional SDS–PAGE. McCormack et al. subsequently reported that binding of SP-A to purified MSG was reduced by deglycosylation of the MSG with *N*-glycanase and blocked by several individual point mutations of the Ca^{2+}- and carbohydrate ligating residues of the CRD [47]. Collectively, the data indicate that the CRD of SP-A binds to *N*-linked carbohydrates associated with MSG, but the existence of other binding epitopes, such as *O*-linked carbohydrate or peptide domains, cannot be excluded. The importance of SP-A in clearance of Pc is supported by recent, preliminary evidence that SP-A deficiency results in enhanced susceptibility to Pc pneumonia in SP-A gene targeted mice [97].

SP-A has also been reported to bind to glycolipids, several of which have recently been identified in extracellular surfactants isolated from rats and humans. SP-A specifically binds to lactosylceramide and galactosylceramide on TLC plates or adsorbed to plastic wells [98]. The structural basis and physiological significance of glycolipid binding by SP-A remains unclear.

4.3. SP-A–phospholipid interactions

4.3.1. Background

Protein–surfactant phospholipid interactions that occur at the oil–water interface constitute an important yet poorly characterized area of pulmonary biology. SP-A is a Ca^{2+}-dependent phospholipid binding and aggregating protein, that is intimately associated with surfactant phospholipids in the alveolar space. The physiological importance of SP-A–phospholipid interactions is not fully understood, but several roles for SP-A in surfactant function have been proposed.

4.3.1.1. SP-A is required for the structure and/or stability of surfactant aggregates. This is one of the more thoroughly substantiated of SP-As surfactant roles. Tubular myelin contains large amounts of SP-A, and SP-A is required to produce tubular myelin forms from purified surfactant components DPPC and SP-B, in vitro [99]. SP-A gene targeted animal models that are deficient in SP-A have little or no tubular myelin [83]. SP-A may drive the formation of tubular myelin in the extracellular space as the lamel-

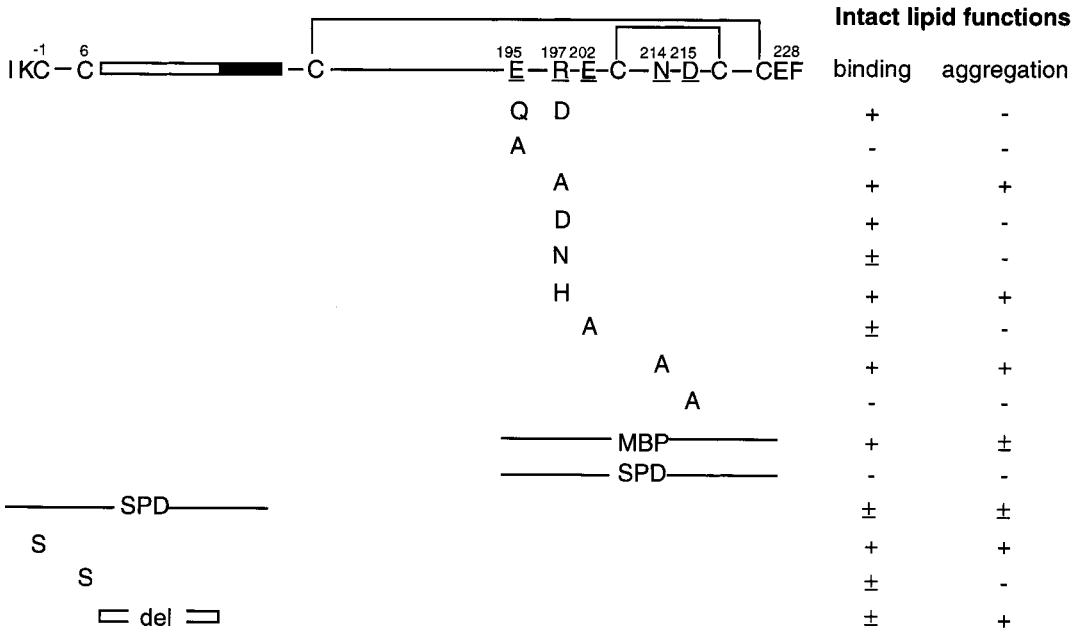

Fig. 3. Mapping of domains of SP-A that mediate lipid binding and aggregation by site-directed mutagenesis. Specific amino acid substitutions and deletions are aligned with a schematic representation of the primary structure of rat SP-A (top left) and with the functional consequences of each mutation (right). Mutations that have been reported include (from N- to C-terminus): individual Ser (S) substitutions of the two disulfide forming N-terminal Cys (C) [29,30], deletion the collagen-like domain (open box) [29], substitution of the SP-D N-terminal segment and collagen domain [113], single [46,47,73] and tandem [45] amino acid substitutions for the SP-A residues E(Glu)195, R(Arg)197, E(Glu)202, N(Asn)214, D(Asp)215 that correspond to the Ca^{2+} coordinating residues of MBP-A (see Fig. 2), and SP-D [74] and MBP [75] CRD sequences that were substituted for the corresponding segments in SP-A. Deletion of the N- and C-terminal N-linked oligosaccharides had no effect on the functions listed (not shown) [71]. The neck domain (closed box) and the large (Cys 135–Cys 226) and small (Cys 204–Cys 218) intramolecular disulfide loops are also shown.

lar body unfolds, or it may stabilize an otherwise transient lattice structure. In vitro cycling studies have also provided evidence that SP-A enhances stability of large surfactant aggregates [100], of which tubular myelin is a member. Low-speed centrifugation of the bronchoalveolar lavage sediments the more surface active large (or dense) surfactant forms, which can be converted to the inactive, less sedimentable, 'light' forms by cycles of surface expansion and compression in vivo (i.e., with respiration) [101] or by tumbling in a football shaped vessel in the laboratory [102]. SP-A retards large aggregate conversion in vitro and in vivo, suggesting that the protein may play a role in maintaining a surface-active surfactant pool [103]. The role of individual structural domains of SP-A in this activity has not been examined.

4.3.1.2. SP-A protects the surface activity of surfactant films. Acute lung injury results in the flooding of the alveolar space with serum proteins that directly interfere with the function of surfactant [104–106]. SP-A blocks foreign protein-induced inhibition in vitro [107] and in vivo [108]. Recently, surfactant isolated from the SP-A gene targeted mouse was shown to be susceptible to protein inhibition [84]. No structural data are available for the mechanism of this SP-A function.

4.3.1.3. SP-A augments the adsorption of phospholipids to an air–liquid interface and improves surface tension of cycled surfactant mixtures, in vitro. Two lines of in vitro evidence suggest that SP-A may play a role in the assembly and refinement of surfactant films. Hawgood et al. reported that SP-A, in concert with the hydrophobic surfactant proteins, had a minor cooperative effect on the adsorption of surfactant phospholipids to an air–liquid interface, in vitro [109]. Secondly, the addition of small amounts of

SP-A enhances the effect of cycling on the reduction in minimum surface tension of surfactant films adsorbed to the surface of an oscillating air bubble [107]. Recent evidence that the collagen-like domain and the CRD are both required for the surface tension lowering effects of SP-A are discussed below. It is not clear that these in vitro surface active properties of SP-A are physiologically relevant in the lung, however. Surfactant replacement reagents which contain no SP-A are effective therapies for infant respiratory distress syndrome [110], and SP-A gene targeted animals have normal lung compliance and only subtle abnormalities in surfactant function [83]. It is possible that SP-A plays a role in the surface tension lowering properties of surfactant which only become manifest in the presence of specific stressors.

4.3.2. The role of individual SP-A structural domains in the phospholipid binding, liposome aggregation and surface tension lowering effects of SP-A

The structural requirements for the specific binding of SP-A to phospholipids have been determined using ligand blots of informative phospholipid species and mutagenesis of the protein (Fig. 3).

4.3.2.1. Phospholipid structural determinants for SP-A binding. SP-A binds avidly to dipalmitoylphosphatidylcholine (DPPC) but less strongly to phosphatidylcholine, indicating that saturated fatty acid side chains are important in the interaction [111]. Sphingomyelin is also a preferred ligand but SP-A does not bind to the other major phospholipid components of surfactant including phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine or phosphatidylserine. Binding is retained by dipalmitoylglycerol but not by palmitic acid or lysophosphatidylcholine, suggesting that two adjacent glycerol-coupled fatty acid chains are required for the interaction. The specificity of the polar head group also plays an important role in the binding, since SP-A does not bind to dipalmitoylphosphatidylglycerol.

4.3.2.2. SP-A structural determinants for phospholipid binding and aggregation. The mapping of the phospholipid interaction domain of SP-A has been addressed in several mutagenesis studies. Early reports suggested that the lipid binding site of SP-A was located in the neck region of the protein, a concept that was consistent with the hydrophobic nature of this domain [112]. The finding that a chimeric collectin composed of the N-terminal region+collagen domain of SP-D and the neck+CRD of SP-A bound to DPPC also supported the existence of a lipid binding site in the C-terminal domains of SP-A [113]. However, characterization of a mutant SP-A with tandem mutations in the CRD (Glu195Gln, Arg197Asp) revealed that the liposome aggregation function of SP-A was lost, implicating the CRD rather than the neck as the major lipid interaction site [47]. This notion was also supported by a study by Kuroki et al., who found that both lipid binding and aggregation were blocked by a monoclonal antibody (denoted ID-6) that recognized a epitope near the C-terminal end of the CRD, but not by a neck specific monoclonal antibody (denoted 6E3) [39]. Subsequent mutagenesis experiments have shown that alanine substitutions for the acidic amino acids that coordinate the Ca^{2+} ion in the carbohydrate binding site of the CRD (Glu195Ala, Glu202Ala, Asp215Ala) block lipid binding and aggregation [46]. Amino acid substitutions for polar (Asn214Ala) and basic amino acids (Arg197Ala) in that site that are also thought to form Ca^{2+} coordination had variable but generally less pronounced effects on lipid interactions. Chimeras made from CRD sequences of SP-D substituted for corresponding segments in the C-terminal end of the CRD of SP-A provided additional information [74]. All SP-A functions and the ID-6 antibody epitope (Cys 204–Cys 218) were lost following even the most conservative substitution, a chimera in which SP-A sequences were replaced with an SP-D segment corresponding to the first Ca^{2+}-coordinating residue of the carbohydrate binding site through the C-terminus. Seemingly conflicting results were obtained when highly functional chimeras were similarly constructed from MBP-A-CRD sequences [75]. However, the MBP-A but not the SP-D peptide segments that were exchanged with SP-A sequences contained all of the critical amino acids for coordination of Ca^{2+} in the same positions as they occur in SP-A (Glu 195, Glu 202, Asn 214, Asp 215). The Ca^{2+} binding site and the ID-6 monoclonal antibody epitope were clearly reconstituted in the SP-A–MBP-A chimera, probably because the

SP-A and MBP-A sequences are sufficiently similar. The functional result was nonetheless surprising, because there are several amino acid differences resulting in charge neutralization and inversion in the reengineered SP-A–MBP-A CRD. Collectively, the results of the point substitution and chimeric mutagenesis studies are consistent with the localization of the major lipid binding site of SP-A to the CRD. The lipid binding site(s) may overlap with Ca^{2+}–carbohydrate binding site, or may be located at another site within the CRD which requires a Ca^{2+}-dependent conformational change to become exposed and/or active. Precise identification of the lipid interaction site is difficult to achieve with mutagenesis experiments alone and will likely require solution of the structure of the SP-A–lipid complex by *x*–ray crystallography, as has been accomplished for the phospholipid binding proteins binding proteins BPI [114] and annexin V [115].

The N-terminal domains of SP-A also play important roles in lipid vesicle binding. Mutant recombinant rat SP-As bearing a deletion of the collagen-like domain or a disrupted N-terminal interchain disulfide bond at Cys^6 bind to liposomes only 1/2 and 1/3 as effectively as the wild-type recombinant protein, respectively [29]. These mutations caused a reduction in oligomeric mass of the proteins as determined by gel filtration, albeit to a lesser degree than was anticipated. Our interpretation of these data is that the binding of SP-A to liposomes is mediated by relatively weak interactions between the CRD and phospholipids at the vesicle–solvent interface, which are amplified by the simultaneous binding of several CRDs from individual oligomers.

The Ca^{2+}-dependent aggregation of phospholipid vesicles by SP-A is a useful model to analyze SP-A's role in the formation and stability of surfactant aggregates and films. The CRD is critical for the lipid aggregation function of SP-A and, in general, the effects of various CRD mutations on the lipid binding and lipid aggregating functions of SP-A closely parallel one another (e.g., Glu195Ala, Glu202Ala, Asp215Ala mutations block both lipid binding and aggregation [46]). However, the finding that the Glu195Gln, Arg197Asp [45] and the Arg197Asp [73] mutant SP-As bind but do not aggregate phospholipid vesicles indicates that lipid binding is not sufficient to cause aggregation. The dissociation of these functions suggests that although the lipid binding and aggregation domains of SP-A may overlap, they are clearly not identical. One possibility is that a specific conformational change in the CRD is required to effect lipid aggregation once binding has taken place, a shift that may be disrupted in the QPD protein. However, recent analyses of the kinetics of SP-A liposome interactions using the resonant mirror technique and near-infrared light scattering supported the sequence; SP-A conformational change, liposome binding, liposome aggregation [116]. Another interesting hypothesis that could accommodate the separation of lipid binding and aggregation domains was suggested by Haagsman et al. [117]. They found that deglycosylation eliminated aggregation by human SP-A, suggesting that crosslinking of SP-A–vesicle complexes occurs by the ligation of *N*-linked oligosaccharides of SP-A by CRD domains from neighboring liposomes. In their model, SP-A must simultaneously bind to liposomes and to itself, though a lectin–oligosaccharide interaction, to effect aggregation. This mechanism may not apply to all species however, since the genetic deletion of carbohydrate from recombinant rat SP-A did not block phospholipid aggregation [71].

The N-terminal domains of SP-A are also important for lipid aggregation [29]. Disruption of the interchain disulfide bond at Cys 6 completely blocks lipid vesicle crosslinking by SP-A. However, deletion of the collagen-like domain without disruption of Cys 6 disulfide bridge does not affect aggregation. These data indicate that Cys 6 interchain disulfide linkage but not the collagen-like region is required for aggregation. Preliminary evidence indicates that the Cys −1 intermolecular disulfide bridge is much less critical than Cys 6 for lipid aggregation [30].

4.3.2.3. SP-A structural determinants for enhancing the surface active properties of surfactant films. SP-A has been shown to reduce the minimum surface tension that surfactant films can achieve when they are cyclically compressed and expanded [107]. Recently, we reported that the Cys 6 intermolecular disulfide bond but not the collagen domain is required for this function [29]. The surface tension lowering effect of SP-A appears to require a cooperative interaction between N-terminal domains and the CRD, since the Glu195Gln, Arg197Asp protein

also has no effect on surface pressure in the laboratory [29]. We speculate that interchain linkage at Cys 6 is required to maintain the oligomeric structure of the protein that optimizes CRD interactions with the phospholipids at the interface, but delineation of the mechanisms involved will require further experiments.

5. SP-A–type II cell interactions

5.1. Background

SP-A regulates the secretion and uptake of surfactant by isolated type II cells [118,119]. Disruption of SP-A–SP-A receptor interactions by biochemical modifications of SP-A or incubation with anti-SP-A antibodies block surfactant regulation by the protein, indicating that the two events are coupled [120]. The inhibitory effect of SP-A on surfactant secretion from isolated type II cells is not blocked by incubation with excess monosaccharides [120]. This property is used as the hallmark of specific inhibition by SP-A, since other unrelated, nonpulmonary lectins have been shown to inhibit surfactant secretion by a carbohydrate-reversible mechanism [121]. Inhibition of secretion is maximal at a concentration of 5–10 μg/ml SP-A [73,118,119], which is at least 15-fold lower than the physiological concentration predicted in the alveolar hypophase [122]. However, almost all SP-A in the extracellular compartment of the lung is complexed with lipid, therefore the availability of the protein for physiological cellular interactions is unclear. Secretagogues increase the numbers of SP-A receptors on the cell surface of isolated alveolar type II cells, suggesting that intracellular pools of receptors are available for recruitment [123]. The existence of a high affinity receptor(s) for SP-A on the surface of type II cells and the coupling of occupancy of the receptor to a specific effect on surfactant secretion support a role for SP-A in the control of surfactant phospholipid levels in the alveolar space. However, an SP-A gene targeted mouse with undetectable SP-A levels had normal surfactant pool sizes under resting conditions [83]. Further studies will obviously be required to determine if SP-A is required for the physiological control of surfactant homeostasis.

Several approaches have been applied to the molecular characterization of the SP-A receptor on the surface of type II cells. Strayer identified a 30 kDa type II cell membrane SP-A recognition protein (SPAR) using anti-idiotype antibodies directed against monoclonal anti-SP-A antibodies [124]. The SPAR proteins have projected structures which are consistent with cell membrane receptors, and are expressed primarily in the lung. The anti-SPAR antibody inhibits SP-A receptor mediated Ca^{2+}-signaling and inhibition of secretion from isolated alveolar type II cells [125,126]. Stevens et al. developed a type II cell antibody by an auto anti-idiotypic antibody technique using SP-A as the antigen [127]. The antibodies recognize a cell surface SP-A binding protein that has an apparent mass of 55 kDa (BP55) under reducing conditions and 170–200 kDa under nonreducing conditions. The antibody competes for the binding of SP-A to type II cells, and blocks SP-A mediated uptake of liposomes by the pneumocyte [128]. Interestingly, the antibody had no effect on the inhibition of secretion of surfactant by SP-A, suggesting that uptake and secretion may be mediated by two separate receptors. Chroneos et al. purified a 210 kDa SP-A binding protein from a U937 macrophage cell line using affinity chromatography with SP-A as the ligand [38]. The 210 kDa protein was also isolated from rat lung membranes using ligand and detergent extraction, respectively. The antibody inhibited the binding of SP-A to type II pneumocytes by 70% and blocked SP-A mediated inhibition of surfactant secretion. Interestingly, the incubation of the anti-SPR210 antibody with isolated type II cells alone appeared to stimulate the secretion of surfactant. The binding of SP-A to macrophages and SP-A mediated phagocytosis of the mycobacterium, BCG, into macrophages were also inhibited by anti-SP-R210 [129]. The tissue distribution of the SP-R210 protein has not been reported. Nepomuceno et al. have cloned and sequenced a 126 000 M_r cell surface protein (denoted C1qRp) from U937 cells that binds to C1q, MBP-A and SP-A [130]. The C1qRp contains several interesting domains including a C-type CRD, five EGF domains, a transmembrane domain and a tyrosine phosphorylation motif. The tissue distribution of this receptor has not been reported and it is not clear if it is expressed on type II cells. This receptor is clearly distinct from a C1q

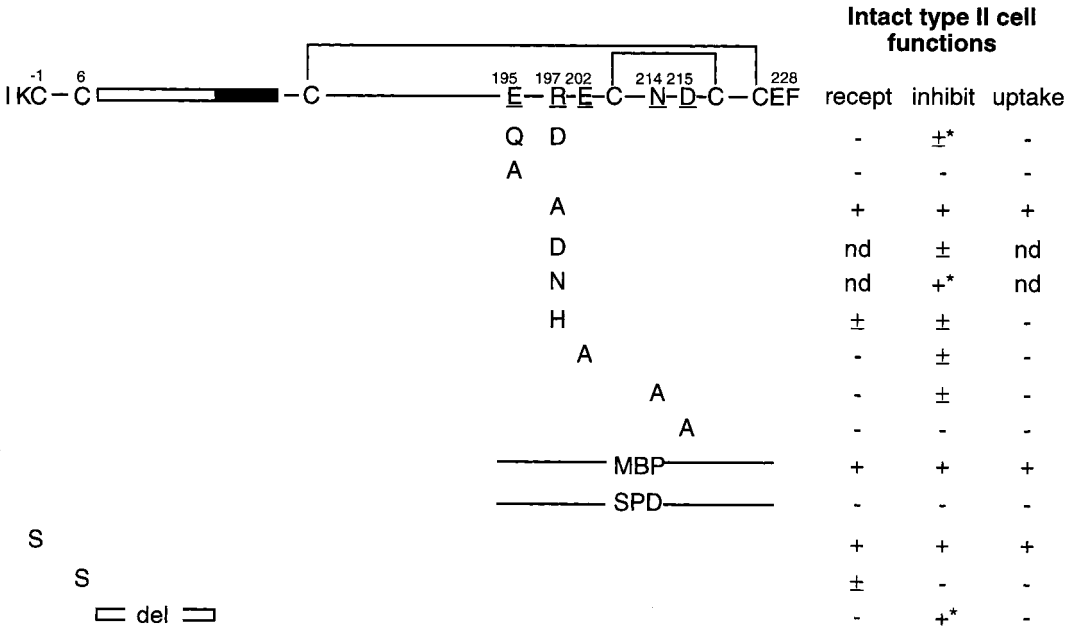

Fig. 4. Mapping of domains of SP-A that mediate receptor binding and regulation of surfactant secretion and uptake into isolated type II cells by site-directed mutagenesis. The functional consequences of various reported mutations (Refs. supplied in legend to Fig. 3) of SP-A on receptor binding (recept), inhibition of surfactant secretion from isolated type II cells (inhibit), and enhanced uptake of liposomes into type II cells (uptake). An asterisk indicates that inhibition of secretion was reversed with excess monosaccharides and therefore nonspecific. Deletion of the N- and C-terminal N-linked oligosaccharides had no effect on function (not shown) [71]. nd, not done.

and SP-A binding protein isolated by Malhotra et al. (C1qR), which has a reduced molecular mass of 60 kDa and significant homology to calreticulin [32,131,132]. The C1qR appears to bind to SP-A and C1q via the N-terminal end of their collagenous regions. Determination of the importance of each of these receptors for pulmonary homeostasis may require ablation by gene targeting.

5.2. *Role of individual SP-A structural domains in the receptor-mediated regulation of surfactant uptake and secretion by isolated alveolar type II cells*

Mutagenesis studies have mapped the SP-A receptor binding and surfactant regulatory functions to the CRD (Fig. 4). The introduction of tandem mutations into the CRD that altered carbohydrate binding specificity (Glu195Gln, Arg197Asp) blocked binding to the receptor and the regulation of surfactant secretion and uptake [45]. The substitution of the Ca^{2+} ligating residues of the carbohydrate binding site with alanine (Glu195Ala, Glu202Ala, Asn214Ala, Asp215Ala) had the similar effects on SP-A's receptor binding and surfactant regulatory functions [46]. Alanine substitution for Arg 197 was much less disruptive, consistent with less conserved nature of this position between SP-As of various species [73]. Interestingly, the substitution of Arg 197 by His resulted in a protein which retained surfactant inhibition and liposome interaction activities but which did not promote lipid uptake. The dissociation of these two functions suggests that there may be subtle differences in the domains of the CRD which mediate inhibition of secretion and uptake of phospholipid vesicles. Chimeras in which portions of the CRD of SP-A were replaced with corresponding sequences from SP-D were inactive [74], but similarly constructed chimeras made from MBP-A sequences competed for receptor occupancy and were effective regulators of surfactant homeostasis [75]. The fact that the MBP-A segments can functionally replace the homologous SP-A sequences is probably related to the preservation of key residues in the Ca^{2+} and carbohydrate binding site. Collec-

tively, these studies indicate that the CRD of SP-A mediates the binding to a receptor population on isolated alveolar type II cells that controls surfactant secretion and uptake.

The domains of SP-A that contribute to oligomeric assembly also appear to play an important role in inhibition of surfactant secretion and uptake. Disruption of the Cys 6 intermolecular disulfide bond blocks surfactant uptake and specific inhibition of surfactant secretion by type II cells [29], but disruption of the Cys −1 interchain bond does not appear to have a major effect [30]. Deletion of the collagen-like region blocks competitive binding to the SP-A receptor, specific inhibition of surfactant secretion and surfactant uptake [29]. Interestingly, deletion of the collagen-like domain does not affect inhibition of secretion in the absence of excess carbohydrates (i.e., nonspecific inhibition). These data suggest that the collagen-like region serves a cooperative role in the interaction with the SP-A receptor, either through a direct protein–protein interaction or by enhancing the valency of SP-A for the receptor. It is also possible that the collagen-deletion mutant form of SP-A interacts with a separate receptor, perhaps the same cell surface molecule that mediates Con A-induced inhibition of surfactant secretion [121].

6. SP-A–lipopolysaccharide interactions

6.1. Background

All higher organisms have developed sensitive mechanisms for immediate response to infection with Gram-negative organisms triggered by the bacterial cell wall component lipopolysaccharide (LPS or endotoxin) [33,34]. While there have been major advances in the understanding of LPS effects in the systemic circulation, very little is known about regulation of LPS activity in the lung. An ideal system would discriminate between LPS exposures that herald a legitimate threat, such as pneumonia, and exposures are not signals for infection, such as LPS inhalation in the absence of bacterial infection. In the systemic circulation, the activity of LPS is regulated by variety of proteins including LPS binding serum factors including LBP [133], sCD-14 [134] and MBP-A [135]; the lysosomal protein BPI [114];

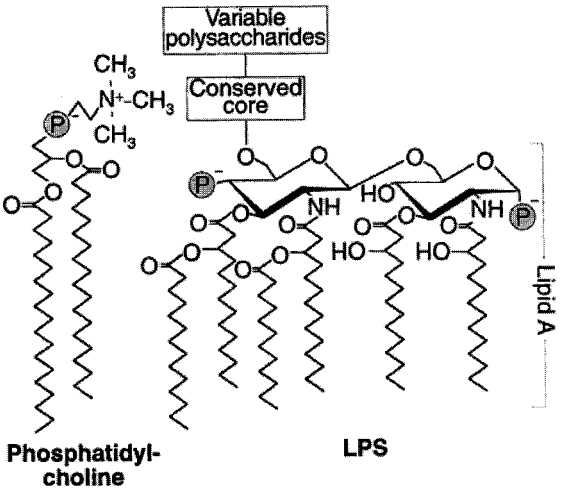

Fig. 5. Lipopolysaccharide structure-LPS contains a hydrophobic lipid A domain and hydrophilic domains composed of conserved core polysaccharides and variable polysaccharides. Note structural similarities with the SP-A ligand, phosphatidylcholine. (Drawing from Ref. [114].)

and the membrane receptor CD-14 [136]. It is reasonable to speculate that LPS activity modifying molecules also exist in the airspace, but they are not likely to be LBP and sCD-14, since these proteins are present in only trace amounts in the normal lung [137]. SP-A has emerged as a potential candidate as an LPS regulatory protein. This hypothesis is based on several observations: (1) SP-A binds avidly to LPS via the lipid A domain [33,34], (2) SP-A exhibits an acute phase response to LPS aerosolization [138] or intratracheal inoculation [139], (3) LPS upregulates the expression of the SP-A receptor on macrophages [140], and (4) SP-A modifies the response of AM to LPS [33,141]. Surfactant protein D is another candidate LPS activity modifying protein in the lung that also binds to LPS [142] (see Chapter XV).

6.2. Role of individual LPS and SP-A structural domains in binding

LPS is a complex glycolipid composed of two distinct domains [143] that may serve as ligands for SP-A (Fig. 5). These include hydrophilic repeating polysaccharides which make up the conserved core and more variable *O*-antigen domains, and the hydrophobic domain called lipid A. The general

chemical structure of lipid A from diverse Gram-negative bacteria is highly conserved and virtually all LPS-induced biological responses are lipid A-dependent [144]. The structure of lipid A closely resembles that of phospholipids such as phosphatidylcholine (Fig. 5), which was incidentally found occupying the LPS binding site in bacterial permeability-increasing protein (BPI), a neutrophilic granule protein that is homologous to LBP [114]. Given that phosphatidylcholine is a known ligand for SP-A, and that LPS contains abundant carbohydrate, it is tempting to speculate that SP-A may bind to one or both of these domains of LPS via the CRD. The finding that SP-A binds to LPS via the lipid A moiety in a specific and Ca^{2+}-dependent manner is consistent with this notion [34], although one laboratory found no Ca^{2+}-requirement [33] and mannan or α-D-mannopyranoside did not inhibit binding of SP-A to LPS. SP-A appears to bind preferentially to rough LPS, which contains lipid A and two sugar residues, and not to the more heavily glycosylated smooth LPS variants [34]. Mutagenesis studies in progress in several laboratories should elucidate the structural elements of SP-A that are required for LPS binding [145].

7. Phospholipase inhibition

Phospholipase A_2 (PLA_2) is a family of enzymes that hydrolyzes the fatty acyl group in the *sn*-2 position of phospholipids. In 1991, the primary structure of a PLA_2 inhibitor from the blood plasma of the Habu snake was isolated and shown to have sequence homology with SP-A [146]. This observation led to a series of experiments to investigate whether SP-A may play a role in the regulation of PLA_2 activity in the lung, especially the synthesis and degradation of surfactant phospholipids in intracellular and extracellular compartments. Fisher et al. demonstrated that SP-A inhibits Habu snake PLA_2 activity, by a mechanism that involves a protein–protein interaction [147]. They also reported that SP-A inhibits a newly characterized Ca^{2+}-independent PLA_2 present in granular pneumocytes and lung lamellar bodies but not PLA_2 isolated from bovine pancreas [148,149]. Since SP-A blocked PLA_2 activity for lipids that are not ligands for SP-A, it is unlikely that the effect of SP-A is due to a ligand sequestration effect as has been demonstrated for other PLA_2 inhibitors. Further studies will be required to determine if PLA_2 inhibition by SP-A plays a physiologically relevant role in the lung, and to define the structural basis of the interaction between PLA_2 and SP-A.

8. Other ligands for SP-A

Annexin IV is a Ca^{2+}-dependent phospholipid binding protein that is enriched in the cytosol of alveolar type II cells. Annexins are thought to play an important role in exocytosis. Recently, annexin IV was shown to be a major SP-A binding protein in the soluble fraction of bovine lung homogenates [150]. The binding was Ca^{2+}-dependent and was preserved even after delipidation of the protein, indicating a direct protein–protein binding mechanism. Lung lamellar bodies also bound annexin IV, suggesting a possible role for an SP-A–annexin IV interaction in lamellar body function. SP-A has also been reported to bind to cellular myosin [151]. The physiological relevance of this interaction is not clear, but may serve to facilitate the clearance of myosin that is shed into the alveolar hypophase by desquamated or decaying cells. Finally, SP-A has been shown to bind to the major outer membrane protein (OMP) of Hemophilus influenza in a Ca^{2+}-dependent manner [87]. The Ca^{2+}-dependent binding of SP-A to non-glycosylated proteins such as OMP suggests that the CRD of SP-A may recognize peptide ligands.

9. Summary and future directions

The abundance of SP-A in the alveolar space and the conservation of SP-A across species attest to the importance of the protein in pulmonary function. Based on the homology of SP-A with MBP-A, data from several laboratories demonstrating opsonic and macrophage activating properties, and the bacterial clearance defect of the SP-A-deficient mouse, it seems clear that SP-A performs a host defense role in the lung. The presence of a high affinity receptor for SP-A on alveolar type II cells, the specific binding of SP-A to surfactant phospholipids, the effects of

SP-A on the assembly, integrity and surface activity of surfactant films, and the demonstrable surfactant function abnormalities in SP-A gene targeted mice suggest that SP-A may also be an important protein for surfactant function. A recurring theme from structural studies of SP-A is that the CRD is the predominant ligand binding domain for Ca^{2+}, carbohydrates, lipids and cell surfaces, and that the N-terminal domains support the multivalent, oligomeric structure required for high-affinity interactions. Advances in the understanding of the structure–function relationships of SP-A will be greatly facilitated by the solution of its crystal structure, both as an apoprotein and complexed with its biological ligands. Ultimately, validation of the physiological relevance of each proposed SP-A function will require testing in natural and genetically engineered animal models.

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